A FRET Study of Guest Delivery to Concanavaline A by Supramolecular Hosts Composed of an Adamantyl-Appended Cyclophane and Saccharide-Branched Cyclodextrins

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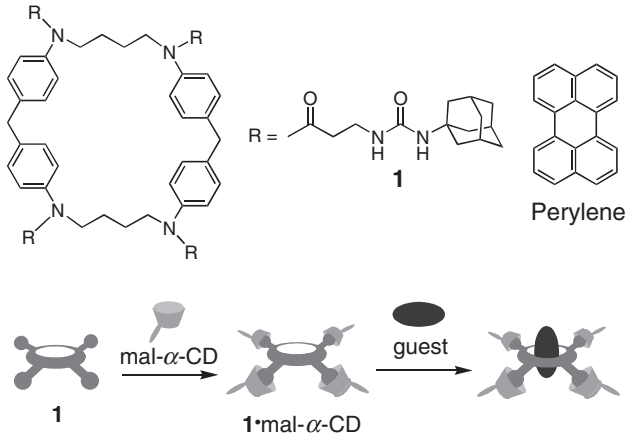
Water-soluble supramolecular hosts composed of an adamantyl-appended cyclophane and glucosyl- or maltosyl-α-cyclodextrins were developed as a transporter for fluorescent guest toward Concanavaline A (Con A), α-D-glucoside-binding lectin. The delivery of perylene as a model guest from aqueous media to Con A surfaces was monitored by fluorescence resonance energy transfer (FRET) spectroscopy.

Supramolecular chemistry is an important strategy to create nanometer-sized cavities[1] for guest molecules as well as assemble ligands[2] for biological receptors. We have recently reported the development of a water-soluble supramolecular host composed of a cyclophane bearing adamantyl moieties (**1**)[3] and α-cyclodextrin (α-CD). The supramolecular host exhibited guest binding affinity toward hydrophobic guests such as 8-anilinonaphthalene-1-sulfonate and 6-*p*-toluidinonaphthalene-2-sulfonate with binding constants of 4.1×10^3 and $1.1 \times 10^4\ M^{-1}$, respectively ($1\ M = 1\ mol\ dm^{-3}$). These guest molecules were incorporated into the internal cavity of **1**, which was well shielded from the bulk aqueous phase. When 6-*O*-α-maltosyl-α-cyclodextrin (mal-α-CD)[4] is assembled with **1** to give a saccharide-functionalized supramolecular host **1**·mal-α-CD as shown in Scheme 1, the saccharide branches of mal-α-CD is expected to act as a ligand directed toward carbohydrate-binding proteins[5] (lectins). In this context, we report here the complexation behavior of the supramolecular host with fluorescent perylene and its delivery system from bulk aqueous media to lectin surfaces monitored by fluorescence spectroscopy.

Cyclophane **1** is scarcely soluble in water but easily dissolves upon addition of a large excess amount of mal-α-CD to give a supramolecular assembly (**1**·mal-α-CD) (Scheme 1). At least within the concentration range of 7 μM–0.05 mM of **1** in the presence of mal-α-CD (10 mM), a good linear Beer's plot of absorbance at 240 nm was observed. Upon complexation with mal-α-CD, induced circular dichroism band was observed in the absorption ranges of **1** through its stereochemical interactions with chiral cavities of mal-α-CD; $[\theta] = -1.8 \times 10^4\ deg\ cm^2\ dmol^{-1}$ at 242 nm. The guest-binding behavior of the resulting supramolecular host (**1**·mal-α-CD) toward perylene was examined by fluorescence spectroscopy in aqueous HEPES buffer (50 mM, pH 7.0) at 298 K. Upon addition of **1** to an aqueous mal-α-CD solution containing perylene, a fluorescence intensity originated from the guest molecule was subjected to increase showing simple saturation behavior (Figure 1). The stoichiometry for the complex was confirmed to be 1:1 host:guest by Job plot (data not shown). The binding constant (K) of **1**·mal-α-CD toward perylene was evaluated on the basis of the Benesi–Hildebrand relationship; $1.3 \times 10^4\ M^{-1}$. The binding affinity of the supramolecular host with perylene is much greater than the corresponding value of mal-α-CD ($K = 10\ M^{-1}$) and comparable to that of tetraaza[6.1.6.1]paracyclophane[6] ($K = 1.2 \times 10^4\ M^{-1}$) in acidic KCl–HCl aqueous buffer (50 mM, pH 2.0) for the identical guest. Furthermore, relatively large fluorescence polarization value (P) was obtained for perylene incorporated into **1**·mal-α-CD compared with that by the guest alone (P values exercised by perylene; 0.15 and 0.41 in the absence and presence of **1**·mal-α-CD, respectively). The perylene was effectively incorporated into the cavity provided by **1** assembled with mal-



Scheme 1. Schematic representation for the formation of supramolecular assembly composed of **1** and mal-α-CDs, and its complex with guest.

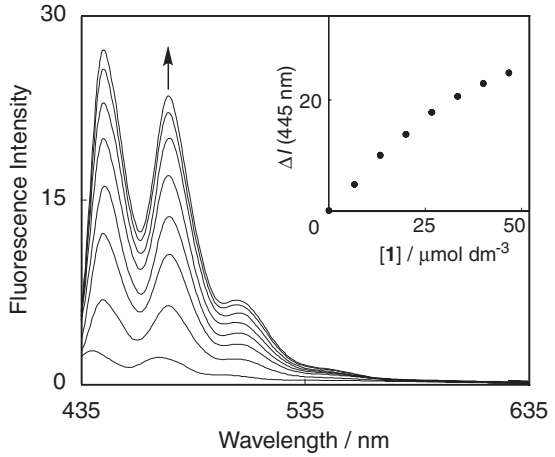


Figure 1. Fluorescence spectra of perylene ($0.5\ \mu mol\ dm^{-3}$) upon addition of **1** in aqueous HEPES buffer in the presence of mal-α-CD ($10\ mmol\ dm^{-3}$) at 298 K. [**1**] (from bottom to top) = 0, 6.7, 13, 20, 27, 33, 40, and $47\ \mu mol\ dm^{-3}$. Ex, 415 nm. Inset; fluorescence titration curve of perylene with **1** in the presence of mal-α-CD.

α-CD. The motional repression of the entrapped guest molecule became effective.

Supramolecular host **1**·mal-α-CD displayed terminal glucoside moieties on the periphery of the complexes, as shown in Scheme 1. We investigated guest delivering ability of **1**·mal-α-CD from bulk aqueous phase to Concanavaline A[7] (Con A), α-D-glucoside-binding lectin, by means of FRET[8] technique. Amplified emission of the entrapped perylene as an acceptor chromophore is expected when the quaternary complex system is excited at the donor absorption of the tryptophan residues of Con A,[9] as shown in Scheme 2. Upon addition of perylene to an aqueous solution containing **1**·mal-α-CD and Con A, a fluorescence intensity originated from tryptophan residues of Con A at 342 nm decreased along with a concomitant increase of the fluorescence intensity of perylene at 445 and 475 nm by the excitation at 305 nm[10] of the tryptophan residues,[11] as shown in Figure 2. A similar fluorescence feature was observed when 6-*O*-α-glucosyl-α-cyclodextrin (glc-α-CD) was employed in place of mal-α-CD for the supramolecular system.[12] On the other hand, the FRET between Con A and perylene did not take place under the conditions described above, but in the absence of **1**. These results suggest that the resulting supramolecular host **1**·mal-α-CD delivered perylene from bulk aqueous phase to the proximal binding sites of Con A surface.

In conclusion, the water-soluble supramolecular host composed of cyclophane **1** with glucosyl- or maltosyl-α-CD demonstrated the potentiality as a transporter. The delivery of perylene from aqueous media to the Con A surfaces by the transporter was monitored by the FRET spectroscopy. We believe that our concept on supramolecular approach in combination with cyclophane cavity and branched CDs provides a useful guidepost for developments of guest delivery systems[13] toward specific lectins, directed by the choice of saccharide units[14] displayed on the periphery on the complexes. Further studies are currently in progress along this line.

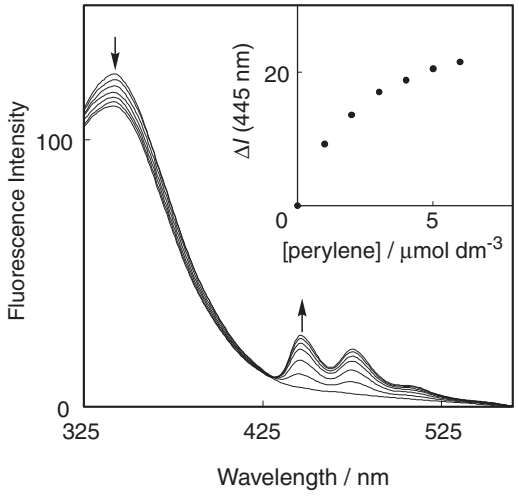
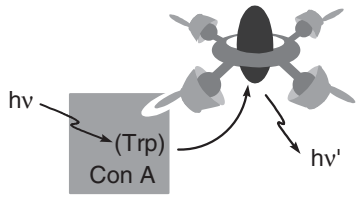


Figure 2. Fluorescence spectral change for aqueous HEPES buffer containing Con A (0.01 mmol dm^{-3}), mal-α-CD (0.5 mmol,dm^{-3}), and **1** (0.05 mmol dm^{-3}) upon addition of perylene at 298 K. Ex, 305 nm. [perylene] = 0, 1, 2, 3, 4, 5, and 6 μmol dm^{-3}. Inset; fluorescence titration curve with perylene.



Scheme 2. Schematic representation for the supramolecular quaternary complex (**1**·mal-α-CD·perylene·Con A) monitored by FRET.

References and Notes

1 a) J. Rebek, Jr., *Chem. Commun.*, **2000**, 637. b) M. Fujita, K. Umemoto, M. Yoshizawa, N. Fujita, T. Kuzuhara, and K. Biradha, *Chem. Commun.*, **2001**, 509.
2 a) S. Sasaki, Y. Shigemasa, and T. Sasaki, *Tetrahedron Lett.*, **38**, 8145 (1997). b) S. Sasaki, Y. Shigemasa, and T. Sasaki, *Bull. Chem. Soc. Jpn.*, **72**, 1313 (1999).
3 O. Hayashida and I. Hamachi, *Chem. Lett.*, **32**, 288 (2003).
4 S. Tanada, T. Nakamura, N. Kawasaki, T. Kurihara, and Y. Umemoto, *J. Colloid Interface Sci.*, **181**, 326 (1996).
5 T. K. Dam and C. F. Brewer, *Chem. Rev.*, **102**, 387 (2002).
6 a) K. Odashima, A. Itai, Y. Iitaka, and K. Koga, *J. Am. Chem. Soc.*, **102**, 2504 (1980). b) K. Odashima, A. Itai, Y. Iitaka, and K. Koga, *J. Org. Chem.*, **50**, 4478 (1991).
7 J. J. Calvete, H. H. Thole, M. Raida, C. Urbanke, A. Romero, T. B. Grangeiro, M. V. Ramos, I. M. Almeida da Rocha, F. N. Guimaraes, and B. S. Cavada, *Biochim. Biophys. Acta*, **1430**, 367 (1999).
8 T. Förster, *Ann. Phys.*, **2**, 55 (1948).
9 T. Heyduk, *Curr. Opin. Biotechnol.*, **13**, 292 (2002).
10 Fluorescence emission of perylene alone in aqueous media by the excitation at 305 nm is negligible.
11 Con A is composed of four subunits in a neutral aqueous solution, each having 237 aminoacid sequences including four tryptophan residues (ref. 5).
12 Decrease in fluorescence intensity at 342 nm was not observed when α-CD was employed in place of mal-α-CD.
13 a) T. Fujimoto, C. Shimizu, O. Hayashida, and Y. Aoyama, *J. Am. Chem. Soc.*, **120**, 601 (1998). b) K. Fujimoto, T. Miyata, and Y. Aoyama, *J. Am. Chem. Soc.*, **122**, 3558 (2000).
14 a) Y. Okada, K. Koizumi, and S. Kitahata, *Carbohydr. Res.*, **287**, 213 (1996). b) Y. Okada, K. Matsuda, K. Koizumi, K. Mamayasu, H. Hashimoto, and S. Kitahata, *Carbohydr. Res.*, **310**, 229 (1998).

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